THE TIDAL RHYTHM OF THE DIATOM HANTZSCHIA AMPHIOXYS

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Several minute chlorophyll-bearing organisms, which inhabit the sand or the mud of the intertidal zone at high tide, migrate to the surface and show photosynthetic activity induced by the sunlight when the tide is low and the beach exposed. At that time they appear on the beach as irregular green, yellow or brown spots.

This behavior is characteristic not only of the well known metazoan *Convoluta roscoffensis*, but also of certain of the Protophyta. Among these are found the Dinoflagellates of the genera Amphidinium, Gymnodinium, Polykrikos (W. A. Herdman, 1911; Storrow, 1913; Whitehead, 1914; Jorgensen, 1918; E. C. Herdman, 1921; Kofoid and Swezy, 1921; Lebour, 1925), the green flagellates of the genus Euglena (Gard, 1919, 1920; Bracher, 1919, 1929; Carter, 1933; Conrad, 1940), the Chrysomonadian *Chromulina psammobia* (Fauré-Fremiet, 1950b), and several diatoms (Bohn, 1904a, 1904b; Fauvel, 1907; Fauvel and Bohn, 1907).

Under natural conditions, the superficial migration of these protophytic organisms appears to be an expression of an intrinsic rhythm which is synchronized with the tide, and which is complicated by the response to light. This rhythm has been studied experimentally in the laboratory, using the following species: the diatom *Pleurosigma estuari* Smith (Fauvel and Bohn, 1907); the green flagellate *Euglena limosa* Gard (Gard, 1919, 1920; Bracher, 1919, 1929, 1938); the Chrysomonadin *Chromulina psammobia* (Fauré-Fremiet, 1950b).

During the past summer a similar behavior was observed in a population of the diatom *Hantzschia amphioxys* Ehrb.,[1] which was found inhabiting the sand of the beach at Barnstable Harbor, Cape Cod.

During the low tide of mid-day in August, irregular spots of different size were visible on the sandy flat near the experimental park of the Woods Hole Oceanographic Institution at Barnstable Harbor. The surface sand, which is slightly muddy, was removed with a spoon and brought to the laboratory for examination. It was then spread over the bottom of large finger bowls and covered with sea water. These cultures were then exposed to the diffuse light from the window.

On succeeding days (the longest period of observation being 6 days), greenish spots similar to those described above re-appeared on the surface of the sand at the time of the low tide at Barnstable Harbor. These disappeared after two to three hours. A microscopic examination showed that the color was due to the superficial accumulation of a great number of the diatom *Hantzschia amphioxys*, associated with a much less numerous undetermined Gymnodinium, and a Euglena.[2]

Hantzschia amphioxys is of rectangular shape, and is slightly asymmetric, having one face convex and the opposite concave. It varies in size from 60 to 80 micra

[1] I wish to express my thanks to Dr. Paul Conger for the determination of this species.

[2] This Euglena resembles *E. limosa* Gard in the lack of flagella and in the metabolic motion, but differs from that form in possessing small and numerous chromoplasts.

in length, and shows two transverse and radiate chromoplasts which are greenish yellow in color. The sliding motion of this diatom is relatively rapid; they travel in the neighborhood of 400 micra in 10 seconds, or about half their total length in one second.

Individuals isolated from the greenish mass exhibit a positive phototropism. When observed under the microscope in a drop of sea water, they move rapidly to the lighted side. This would explain the superficial dispersion of the diatoms both under natural and experimental conditions.

After a few hours, a similar test shows that the phototropistic sign is reversed, the response then being negative. This being true, it would be expected that the diatoms, in the natural habitat, would move into the shadow of the sand grains, leading to a dissolution of the colored spots. The diatoms then have invaded the deeper sandy layers.

During this photophobic phase, the Hantzschia agglutinate among the sand grains. This may well explain why the rising tide does not wash the diatom population away, and has implications of a great general ecological interest.

The observations on the diatoms made in the laboratory with unwashed sand do not permit an exact appreciation of the end-points in the tidal rhythm. Due to the pale color of *Hantzschia amphioxys*, initial phases in surface accumulation and spreading are difficult if not impossible to recognize, and the same is true of the final stages in the disappearance of the surface masses. The behavior of this diatom presents, therefore, a statistical aspect.

It is certain, however, that this species exhibits a physiological rhythm, which is marked by the periodic reversal of its phototropic sign, and by the agglutinative capacity which characterizes the photophobic state.

This physiological rhythm, when observed *in vitro*, is not directly determined by the periodicity of the tide, although it appears to be synchronized to it. In combination with the direct action of light, it controls the rhythmic behavior of the diatoms under the artificial laboratory conditions, and disappears after a few days.

All these facts are in complete agreement with the results of known experiments on the tidal rhythm of *Euglena limosa*, of *Chromulina psammobia,* and of a ciliate associated with symbiotic Chlorella, namely, *Strombidium oculatum*. In the last case (Fauré-Fremiet, 1948), the living conditions are quite different from those of the sand-dwelling species. The above observations lead to the conclusion that the explanation previously suggested for the behavior of the three forms cited above will also apply in the case of *Hantzschia amphioxys*.

Numerous observations and experiments have been made on the rhythmic behavior of simple physiological events. With respect to those which are found in the Protista, as are those described above, it has been presupposed first, that the physiological rhythm is endogenous, and second, that the periodicity which appears to be exactly defined at the population level, is not necessarily so defined in the individual. In addition, it has been assumed that the approximate period for the complete cycle slightly exceeds the 24 hours of a day.

If this is true in the present instance, each rising tide will cause every individual which, at that moment, is not sheltered and agglutinated in the deep layers, to come out of the sand. In other words, a selective elimination takes place at each flood tide, and hence, by elimination of all the "non-conformist" individuals, the synchronism of the endogenous physiological rhythm with the tidal periodicity is maintained.

When this selective elimination is suppressed, as under continued laboratory conditions, the individual variations in periodicity are asserted, and the resulting progressive overlapping of the endogenous periods leads to the disappearance of the collective rhythm.

The statistical maintenance under natural conditions of a synchronism of physiological rhythm and tide is responsible for the apparent adaptation which assures the permanence of a population of small and moving individuals, in a biotope periodically washed by the sea.

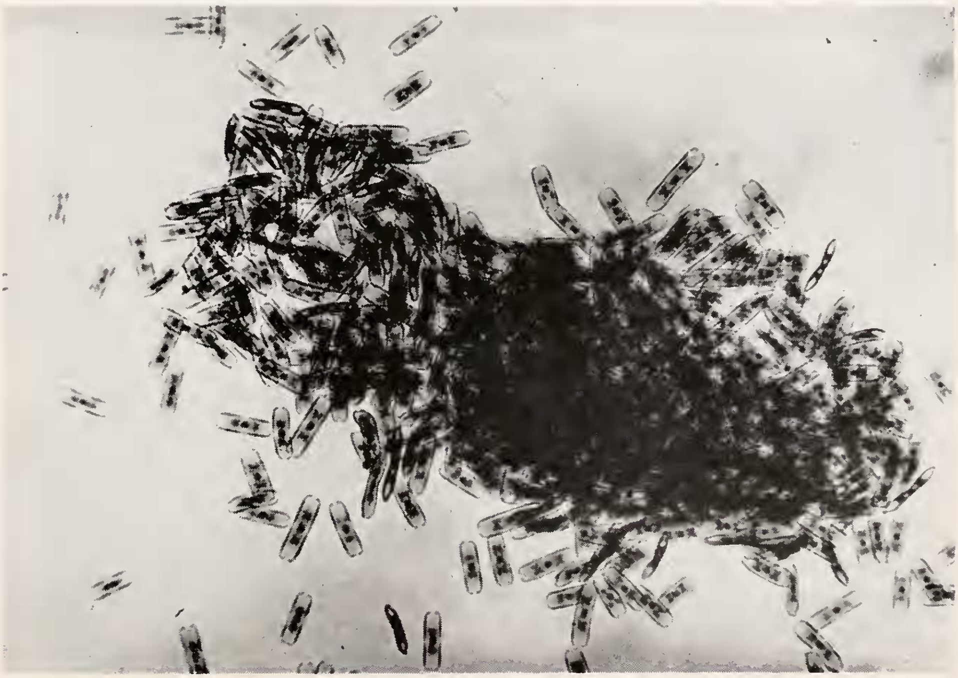

FIGURE 1. A clump of *Hantzschia amphioxys* at the photophobic state, agglutinated on a glass slide under a sand grain layer.

As has been stated above, individuals of *Hantzschia amphioxys* agglutinate to form small clusters in the sub-surface sand when in the photophobic phase. When the same phenomenon is observed in sand on the bottom of a small glass dish or on a microscope slide placed on the bottom of a Petri dish, it may be demonstrated that these clusters adhere to the glass and to the grains of sand. The masses maintain their form and prove to be elastic when they are teased with needles. When fixed while adhering to a slide, it is possible to stain and wash the preparation without freeing the clusters (Fig. 1).

It would appear, therefore, that in the photophobic state, the diatoms are surrounded by a jelly, coagulated by fixative reagents, and possibly a mucoid, which may be considered as a secretion product. These facts are very suggestive in the light of the common statement that slow coagulation of organic substances plays a role in the flocculation and stabilization of marine sediments.

Many interstitial organisms, such as nematodes, copepods, ciliates, and even the agglutinated diatoms themselves, however, continue to move in the sand. The motion is often sufficiently extensive to result in slight displacements of sand grains which are bound together by diffused organic products. Thus, in spite of a relative fragility, the superficial layers of the sand comprise a relatively stable and coarse framework within which there dwells a permanent interstitial microscopic fauna and flora. This is a fact of some ecological interest.

Marine littoral sands represent peculiar and interesting biotopes with respect to the interstitial microfauna. The comparative study of the sand-inhabiting ciliates of the beaches of Brittany and of Cape Cod (Fauré-Fremiet, 1950a, 1951) shows that some species are closely adapted to the interstitial conditions. When free in the water, or when adjacent surfaces are clean solids, the animals prove to be very fragile. The sand grains of the beach must represent a very complex biotope, for the organic substances which cover them appear to have a protective effect on the unstable cytoplasmic surface of the ciliates. At the same time, the organic substances may cohere to render the "structure" of the superficial sand layers more stable.

It is obvious that the secretory and excretory products of the many sand-inhabiting annelids and molluscs play an important part in the development of sand structure. The above observations show that the same is true for many much smaller organisms; among these are the Protophyta and most certainly the bacteria.

I wish to express my thanks to Dr. L. Hoadley for his kind revision of my manuscript.

Summary

1. A sand-dwelling population of the diatom *Hantzschia amphioxys* Ehrb. is described, which exhibits a tidal rhythm that persists for some days when held without tide under artificial conditions in the laboratory. This rhythm is characterized by a periodic inversion of the phototropistic response, and by an agglutination of the members when in the photophobic state.

2. A comparison of this behavior with that of *Euglena limosa*, *Chromulina psammobia* and *Strombidium oculatum* shows close analogies and suggests that the same explanation may account for the synchronization of physiological rhythm and tide in all cases. It seems to be that the rising tide acts as a selective factor.

3. Organic substances produced by the diatoms contribute to the structural stabilization of the superficial sandy layer, and the presence of this organic material appears to be an ecological condition essential to the interstitial microfauna.

LITERATURE CITED

BOHN, G., 1904a. Périodicité vitale des animaux soumis aux oscillations du niveau des hautes mers. *C. R. Acad. Sci.*, **139**: 610.

BOHN, G., 1904b. Oscillations des animaux synchrones de la marée. *C. R. Acad. Sci.*, **139**: 646.

Bracher, R., 1919. Observations on *Euglena deses*. *Ann. Bot.*, **33**: 28–52.
Bracher, R., 1929. The ecology of the Avon banks at Bristol. *J. Ecology*, **16**: 35–81.
Bracher, R., 1938. The light relations of *Euglena limosa* Gard. *J. Linnean Soc., Botany*, **51**: 23–41.
Carter, N., 1933. A comparative study of the algal flora of two salt marshes. II. *J. Ecology*, **21**: 128–208.
Conrad, W., 1940. Sur une Euglene du psammon de l'Escaut. *Bull. Mus. Roy. Hist. Nat. Belgique*, **16**, No. 29.
Fauré-Fremiet, E., 1948. Le rythme de marée du *Strombidium oculatum*. *Bull. Biol. France-Belgique*, **82**: 3–23.
Fauré-Fremiet, E., 1950a. Ecologie des Ciliés psammophiles littoraux. *Bull. Biol. France-Belgique*, **84**: 35–75.
Fauré-Fremiet, E., 1950b. Rythme de marée d'une Chromulina psammophile. *Bull. Biol. France-Belgique*, **84**: 207–214.
Fauré-Fremiet, E., 1951. The marine sand-dwelling ciliates of Cape Cod. *Biol. Bull.*, **100**: 59–70.
Fauvel, P., 1907. A propos du rythme des marées chez les Diatomées littorales. *C. R. Soc. Biol.*, **62**: 242.
Fauvel, P., and G. Bohn, 1907. Le rythme de marée chez les Diatomées littorales. *C. R. Soc. Biol.*, **62**: 121–123.
Gard, M., 1919. Biologie d'une nouvelle espece d'Euglène (*Euglena limosa*). *C. R. Acad. Sci.*, **169**: 1423.
Gard, M., 1920. Recherches sur une nouvelle espece d'Euglène (*Euglena limosa*). *Bull. Soc. Bot. France*, **69**: 184–250; 241–250; 306–312.
Herdman, W. A., 1911. On the occurrence of *Amphidinium operculatum* in vast quantity at Port Erin, Isle de Man. *J. Linnean Soc., Zoology*, **32**: No. 212.
Herdman, E. C., 1921. Dinoflagellates and other organisms causing discoloration of the sand. I. *Trans. Liverpool Biol. Soc.*, **35**: 59–63.
Jorgensen, O., 1918. Occurrence of *Amphidinium operculatum* at Cullercoats. *Rep. Dove Mar. Lab. for 1918.*
Kofoid, C. A., and O. Swezy, 1921. The free-living unarmored Dinoflagellata. *Mem. Univ. of California*, **5**: 1–534.
Lebour, M., 1925. The Dinoflagellates of northern seas. *Plymouth Mar. Biol. Assoc.*
Storrow, B., 1913. Faunistic notes. *Rep. Dove Mar. Lab. for 1912, N. S.*, **2**: 99.
Whitehead, T., 1914. Faunistic notes. *Rep. Dove Mar. Lab. for 1913, N. S.*, **2**: 107.